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Changing Pathology with Changing Drugs: Skin Cancer

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Abstract
Today skin cancer is mainly treated by surgical interventions. New findings concerning molecular biology and the signaling pathways in epithelial skin cancers such as basal cell carcinoma, squamous cell carcinoma or melanoma, and mesenchymal skin cancers such as angiosarcoma and dermatofibrosarcoma protuberans (DFSP) have identified new molecular targets for a systemic or local treatment approach. For DFSP there is an opportunity already today to reduce the intensity of surgical procedures by pretreatment with targeted therapy. This article highlights important aspects in several skin cancer types.

Introduction

Skin cancer is more common than all other cancer forms in human beings. Virtually every single cell type in the skin can transform and start uncontrolled proliferation. In the last decades, intensive research in the field of molecular biology has identified several key events that contribute to the molecular pathogenesis of skin cancers including basal cell carcinomas (BCCs), squamous cell carcinomas (SCCs), and others. Interestingly, there are a number of new therapeutic strategies that are able to specifically target the affected pathways.

Smoothened-Targeted Therapy in BCC

Molecular Biology of BCC

BCC is one of the most common neoplasms in the Caucasian population. Molecular events that are seen in these tumors are rather monotypic in comparison to other malignancies, with translocations and inversions involving 9q being a common aberration in BCCs [1–3]. BCCs rarely develop metastases and are considered to be genetically stable. The high level of stability associated with BCC has been confirmed using comparative genomic hybridization [4] and loss of heterozygosity [5] analyses which showed a reoccurrence of loss on 9q (in 30–60% of tumors), further refining this region as 9q22. Other aberrant loci present in more then 10% of the cases and detected in these and similar studies using molecular-based cytogenetic approaches included chromosome arms 1q, 6p, 6q, 9p, 17p, 17q, and chromosome X [4, 6–9].

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The majority of BCCs are sporadic, but there is a congenital syndrome, i.e. basal cell nevus syndrome (BCNS), which promotes tumor onset. The main promotion of the development of BCCs is provided by sun exposure; however, this relation is complex. Extensive work was done to find predisposing epidemiological factors to explain this tight relation between the environment and BCC development. Among the contributing polymorphic alleles known to date one can name cytochrome P-450 (CYP2D6 EM genotype), glutathione S-transferase-null genotypes (GSTT1 and GSTM1), GSTM3, vitamin D receptor, and tumor necrosis factor gene polymorphisms [10–18]. Very recent association studies of Eastern European and Icelandic cohorts of BCC patients detected fair skin haplotypes of ASIP and TYR loci to increase the risk of BCC development as well as single nucleotide polymorphism variants on 1p36 and 1q42 loci. These new discoveries can point to new targets for BCC chemotherapy [19, 20].

Somatic mutations were found in several genes involved in the Hedgehog (HH) signaling pathway. The first described were mutations in patients with BCNS found in the patched homologue 1 *(PTCH1)* gene located on chromosome 9q22 [21–24]. BCNS was described by Robert Gorlin in the middle of the last century and is often presented as the development of multiple (tens to hundreds) BCCs in an affected patient and at a young age [25]. Patients with BCNS also have a higher risk of developing other types of malignancies, especially medulloblastomas (MB) and radiotherapy-induced BCCs [6–8]. PTCH1 is a human homolog of the patched (ptc) gene in *Drosophila melanogaster* and functions as an inhibitor of the HH signaling pathway. Mutations in this gene lead to constant upregulation of the pathway which is crucial in the development of all BCCs [26, 27]. PTCH1 is not the only gene in the mutation spectrum of BCC which includes genes that regulate skin color, members of the phosphoinositide 3-kinase (PI3K)-Akt and the Wnt pathways, FOXM1 and DNA damage repair genes, and TP53 [28–30]. Furthermore, about 10% of tumors carry mutations in the gene smoothened (SMO), another important member of the HH pathway encoding a regulator downstream of PTCH1 [31–34].

Definition of the above-mentioned events in the molecular pathogenesis of BCC has led to the well-accepted conclusion that upregulation of the HH signaling pathway is essential to promote BCC initiation. Therefore, this signaling pathway has been chosen as a privileged target for the development of molecular interventions.

HH Pathway

Elucidation of molecular aberrations in BCCs enabled the subsequent characterization of a group of human cancers where disturbances of the HH signaling pathway govern tumor development.

In brief, a family of extracellular ligands with 3 members, i.e. Sonic Hedgehog (SHH), Desert Hedgehog (DHH), and Indian Hedgehog (IHH), initiates the HH signaling cascade. All 3 protein ligands are able to bind Patched 1 (PTCH1), a 12-pass transmembrane receptor protein which releases its repression of the pathway activity upon binding of a ligand. PTCH1 suppresses the HH signaling pathway through the inhibition of the G protein-coupled receptor-like protein Smoothened (Smo), which serves as a pathway activator. Through a range of protein interactions Smo transmits a signal to the genes amplified in glioblastoma (Gli) family of transcription factors. When the signal reaches the Gli transcription factors, Gli1, Gli2, and/or Gli3, changes in transcription can then lead to cell proliferation and differentiation [35, 36]. Interestingly, stem cells express a lot of genes that are transcriptionally regulated by Gli 1, 2, and/or 3, thus providing a link between the HH signaling pathway and stem cell function. A short list of target genes involves Gli1, PTCH1, D-type cyclins, BMI1, and Bcl2. Included amongst the transcriptional targets of HH signaling in BCCs are the pathway's own members: *PTCH1*, forming a negative feedback that dampens the pathway; *GLI1*, providing a positive feedback for the pathway, and *HHIP*, which encodes an HH-binding protein [37, 38].

The HH pathway activity is important for the development and homeostasis of several organs and tissues [39]. The physiological mechanism of HH signaling is paracrine; however, during carcinogenesis it is suggested that an autocrine mechanism may be involved [40]. The development of several tumor types is stimulated by mutations in PTCH1 or downstream Smo proteins. Thus, germ line mutations in PTCH1, within manifestations of BCNS, lead to the development of medulloblastoma, ovarian cysts, and ovarian carcinoma. In terms of incidence, mutations in Ptch and/or Smo genes leading to activation of the HH pathway are found in more than 70% of sporadic BCC [32, 41] and in 20–30% of MB [42, 43].

Smoothened-Targeted Compounds

Elaboration of small molecule antagonists of Smo was encouraged by the elucidated and clear molecular pathway of tumor development in BCCs. Since the most prevalent mutations in BCC are activating mutations in

PTCH1 and SMO genes, HH signaling becomes ligand independent in these tumors. Thus, blockade of ligand binding will not result in a therapeutic effect, and inhibition must be targeted to Smo and downstream signaling molecules.

The first Smo-targeted inhibitors, i.e. cyclopamine and jervine, were isolated from corn lilies *(Veratrum californicum)* as teratogenic and antitumor agents [44–46]. However, the chemical structure of these plant alkaloids resulted in low-affinity binding to the Smo protein and decreased bioavailability. This fostered the development of new potent synthetic modifications such as HhAntag [47], SANT1-SANT4 [46, 48], Cur-61414 [49], GDC-0449 [50], and very recently IPI-926 [51]. Several of these inhibitors were able to prevent in vivo tumor progression in MB models driven by mutations in Ptch [52, 53]. GDC-0449 is a new-generation, orally administrated, synthetic Smo inhibitor possessing higher potency and specificity. In a recent phase I trial, systemic administration of GDC-0449 showed promising tumor regressions in metastatic or locally advanced BCC [54]. With respect to clinical testing, very few Smo inhibitors have been used to date in humans. The pioneer is cyclopamine, which is reported to induce the regression of sporadic human BCCs after topical application and inhibit the xenograft growth of HH-overexpressing tumors [40, 55–59]. Another molecule tested topically in humans is Cur-61414, but this promising synthetic Smo agonist did not penetrate human skin well (http://www.curis.com/news.php). The problem of skin penetration is a recurrent obstacle for topical drug application. Presently several strategies are being pursued in order to overcome this issue. One of the actively explored possibilities for cancer therapy is the use of nanoparticles to deliver drugs under the stratum corneum. Some advances have been made recently with the development of solid lipid nanoparticles and core-multishell nanoparticles which can penetrate the skin with high efficiency [60–62]. More promising results, as has already been mentioned, have been obtained using systemically administrated GDC-0449. This molecule, as well as IPI-926, is now entering further clinical trials, is well tolerated, and has very limited acute and chronic toxicity [63].

The current active development of therapeutic agents targeting Smo opens several promising therapeutic avenues. Most promising for the treatment of restricted areas is topical application, as has been shown for cyclopamine, thus avoiding systemic exposure. Points for consideration in this respect are: (1) the long-term complete clearance rate, (2) the types of BCCs responding to treatment (superficial BCC vs. nodular BCC vs. aggressive infiltrative BCC), (3) the time period required to reach tumor clearance, and (4) local side effects and the cosmetic outcome. Patients with a genetically determined high incidence of BCC, e.g. xeroderma pigmentosum or BCNS patients, can profit from systemic therapy. New molecules might be investigated in these patient populations as monotherapeutic tools and in the context of chemoprevention. If successful, such new Smo-targeting drugs could then be investigated in patients with multiple sporadic BCCs. Mounting evidence from mouse models of BCC, as well as early experiences with local and systemic utilization of SMO antagonists, indicates that molecular interventions inhibiting the HH signaling pathway are promising approaches for the treatment of BCC. It is also important to note that there is no evidence that mutation screening is needed before enrolling patients for such a treatment because the vast majority of mutations found in BCCs are mutations leading to HH pathway activation in a ligand-independent manner. These include inactivating PTCH1 mutations leading to the lack of pathway repression (approximately 90% of mutations) and activating SMO mutations leading to constant further signaling (~10% of mutations); mutations in the HH ligand itself are also described [33, 34, 63–66]. However, mutations occurring during treatment may be a concern. As it has been reported for MB, GDC-0449 appears to be able to induce tumor mutagenesis leading to chemoresistance. Therefore, combination therapy will be investigated with agents interfering with molecules downstream of the HH pathway, such as TGF-β, MAPK, or PI3K inhibitors [67–70].

The great need for well-tolerated local and systemic treatment options in patients with multiple BCCs is explained by the continuously increasing incidence of these tumors. It is still unclear which mode of administration, systemic or local, will yield the best efficacy-to-risk ratio. This parameter will determine the place for Smo-targeted therapy in a competitive environment of BCC treatment options alongside surgical procedures, physical therapy (irradiation or photodynamic therapy), and topical immune modulation.

Currently Used Drug Therapy for BCC

There are currently several options available for the treatment of BCCs, e.g. 5-fluorouracil (5-FU), a pyrimidine-like inhibitor of thymidylate synthase which prevents DNA synthesis in tumor cells. The exact mechanism of action in BCC remains unclear for this drug, but it has been suggested that 5-FU downregulates HH path-

way target molecules at both the messenger RNA and protein levels. Interestingly, upregulation of the Gli1 transcription factor restores cell viability and migration inhibited by 5-FU [71].

Based on our improved understanding of the molecular genetics of BCCs, retinoids, especially tazarotene (Tazorac; Allergan), are promising tools for prevention or therapy. Tazarotene has been shown to decrease Gli1 expression and upregulate CRABPII, a target gene of retinoid signaling. Retinoids act through the transcription factors RAR and RXR initiating cellular differentiation and apoptosis as well as extracellular matrix synthesis. They are widely used to treat inflammatory (such as psoriasis or chronic eczema) or neoplastic diseases including acute promyelocytic leukemia, cutaneous T-cell lymphomas, and non-melanoma skin cancers. In Ptch1+/– mice a controlled chemoprevention trial demonstrated that the topical use of tazarotene inhibits the formation of BCCs induced with either UV or ionizing radiation [72, 73]. The total complete clearance rate for BCCs was, however, only 30.5%; therefore, topical tazarotene is not a reliable therapeutic option. In contrast, the substance might be useful as a chemopreventive topical approach based on the data derived from animal models. Prospective trials in humans are testing this hypothesis.

A potent therapeutic agent for the treatment of selected BCCs is imiquimod, a low-molecular-weight synthetic immunomodulator which can induce up to 87% of the clinical and histologically verified clearance of superficial BCCs. One of the major antitumor actions of imiquimod is the induction of inflammation resulting in an IFN-α-driven immune response which has also been confirmed in a mouse model of melanoma [74]. Molecularly this is achieved through triggering of the Toll-like receptor 7/MyD88/NF-κB pathway and the consequent induction, synthesis, and release of selected cytokines, including IFN-α by plasmacytoid dendritic cells. This ability of imiquimod mimics a well-documented treatment of BCC with repeated intratumoral injections of IFN-α [75]. Simultaneously, imiquimod promotes the migration and activation skin Langerhans cells to the regional lymph nodes, Bcl-2-dependant apoptosis of tumor cells and suppresses feedback mechanisms limiting inflammatory responses [76]. How much these pathways contribute to tumor repression in vivo is still undefined [77, 78]. Another interesting phenomenon is the preferential upregulation of Jagged1 protein after treatment with imiquimod. Jagged1 plays an important role in the differentiation of keratinocytes as activation of the Notch pathway triggers terminal keratinocytes differentiation [79].

Epidermal Growth Factor Receptor-Targeted Therapy in SCC

SCC is another very common type of cutaneous malignancy and the second most common cancer in the European population. The increase in its incidence, along with the incidence of BCC, is also attributed to the extent of UV irradiation acquired during a personal lifespan [80, 81]. Quite often advanced-stage SCCs occur in elderly patients and the use of systemic chemotherapeutic agents in such cases represents a certain clinical problem. Therefore, the development of safe and specific molecular-targeted therapies is also of great importance here. A good candidate for such development is epidermal growth factor receptor (EGFR), which is considered to be widely expressed on the surface of SCC cells. Immunohistochemical evaluations of EGFR expression revealed positivity for 40–100% of SCC tumor samples [82–84]. Since phosphorylation of EGFR is associated with the activation of downstream signaling, evaluation of the phosphorylated form of EGFR was performed by Fogarty et al. [85] and the rate was 25%.

EGFR is an important regulator of tumor progression and proliferation in several types of cancer. Preclinical and clinical tests are now focused on 2 types of EGFR-inhibiting strategies: (1) anti-EGFR monoclonal antibodies that block its interaction with endogenous ligands (e.g. EGF and TGF-α) and (2) small-molecule tyrosine kinase inhibitors that inactivate EGFR. Using either approach enables the discontinuation of signal transduction downstream of the EGFR and results in the arrest of tumor proliferation and spread [86–88]. Several drugs, which are products of both development strategies, have been clinically tested in recent years. Examples are: cetuximab, panitumumab, erlotinib, and gefitinib. Cetuximab yielded quite promising results in a phase II multicenter study evaluating its potential as a first-line monotherapy for patients with unresectable SCC. These results were presented at the annual ASCO meeting in 2008 and showed that 77% of patients had stable disease under cetuximab therapy and 22% of patients had a partial response [89].

Despite the promising response rates among non-small cell lung cancer patients who carry somatic mutations in EGFR (the incidence is about 10–15% of the Caucasian population and 30–40% of the Asian patient population), medical problems persist since a large number of patients develop resistance to EGFR inhibitors [90]. In this respect, description of a signaling crossover between HH and EGF pathways in brain, prostate, and skin cell models opens new horizons for therapeutic strategies.

JUN is primarily a transcription activator and its function can be regulated at different levels and depends on phosphorylation by JNK, a target of the MAPK pathway [reviewed in 91]. A suggested mechanism of HH-EGF pathway interaction is through the EGFR-mediated activation of MEK/ERK and JUN cascades and subsequent stimulation of JUN/AP-1 binding with promoters of GLI and ERF target genes, which lead to cancer transformation and invasive growth [92–96]. An interesting recent publication reported the successful use of cetuximab and the COX-2 inhibitor celecoxib in an elderly patient [81]. Such a complex interaction of various molecular pathways provides further opportunities for the development of combination therapies of SCC treatment using a complete range of known drugs, including MEK/RAF/RAS inhibitors, among others.

Somatic mutations in EGFR are reported in about 10% of non-small cell lung cancer cases. Data about the mutation rates in SCC are missing; however, knowledge of the mutation status in SCC patients may be important for response prediction, e.g. in relation to deletion in exon 19. At present, no additional molecular knowledge is needed to start anti-EGFR therapy in SCC as opposed to lung cancer [97]. Nevertheless, it should be noted that SCC histology is associated with a better response to anti-EGFR therapy in esophageal cancer [98]. A more significant issue is acquired chemoresistance to EGFR inhibitors which can occur through mutations in EGFR, KRAS, and NRAS or through MET amplification, which leads to RAF/MAPK/ERK or PI3K/AKT pathway activations [97, 98]. This phenomenon raises a question regarding the better performance of multikinase inhibitors versus immunotherapeutic agents but, unfortunately, clinical data to be able to privilege one class over the other are lacking at present.

Targeted Therapy in Melanoma

Melanoma is a highly aggressive malignancy originating from melanocytes. Most commonly melanoma occurs on the skin, and its incidence rate is constantly increasing worldwide. Presently, it is estimated that around 20 out of 100,000 persons per year will develop a melanoma and estimated lifetime risk for this diagnosis, e.g. in Central Europe, is as high as 1:70 [99]. The majority of affected patients are between the ages of 50 and 60 years, but one fifth of patients are under the age of 40. Active research recently revealed a number of common genetic aberrations in melanoma; these include BRAF and RAS mutations causing activation of the MEK-kinase signaling pathway as well as deletions and amplifications in the genome [99, 100]. However, to date, the therapeutic arsenal lacks efficient treatment modalities that can prolong overall survival rates in patients with metastatic (stage IV) melanoma, and there has been no significant change in the mortality rate associated with melanoma over the past years.

Finding effective therapeutic options to treat melanoma has been an ongoing challenge over the past several decades. Because melanocytes originate from highly motile cells, they are thought to have a high potential for enhanced survival. In vivo, melanoma cells show low levels of spontaneous apoptosis, and in vitro resistance occurs against drug-induced apoptosis [101]. Regarding therapeutic options, a distinction between melanoma subtypes according to their genetics and biological behavior must be made [102–107]. For instance, superficial spreading melanomas, which usually occur on areas of the skin that are not regularly exposed to UV light, are prone to mutations in the BRAF molecule which leads to the activation of signal transduction via RAS and MEK/MAP/ERK-kinase pathways [107]. Bastian et al. [102, 103] recently detected a distinct pattern of chromosomal aberration specific to acrolentiginous as well as mucosal melanomas, thus emphasizing the importance of distinguishing different melanoma types, especially in the context of the increasing number of investigative or registered targeted treatment options currently available. Acrolentiginous melanoma is a rare entity accounting for 2–3% of melanoma [108]; mucosal melanoma only accounts for 0.03% of all melanoma types [109]. Such genetic heterogeneity underlines the necessity of performing genetic evaluations of melanoma patients (i.e. for the presence of the BRAF or RAS mutation) in order to choose the correct chemotherapeutic and/or targeted agent for each subgroup and poses the new challenge of personalized treatment protocols.

RAS/RAF Inhibitors

BRAF mutations are present in over 60% of melanoma biopsies. About 90% of BRAF mutations in melanoma reveal a substitution of valine to glutamic acid at position 600, the V600E mutation [110]. Another 15–30% of melanoma samples carry mutations in NRAS, most commonly induced by a leucine-to-glutamine substitution at position 61 [111].

BRAF and NRAS mutations are mutually exclusive, and consequently 75–90% of melanomas carry activating mutations in the Ras/Raf/MEK/ERK MAPK pathway. At the end of this pathway, ERK is phosphorylated and activates transcription factors implicated in tumor develop-

ment. Furthermore, V600E BRAF stimulates vascular endothelial growth factor (VEGF) secretion, promoting angiogenesis [112, 113]. NRAS also activates the PI3K pathway, leading to proliferation and invasion [114]. Although mutated BRAF and NRAS are also frequently found in benign nevi, nevi lack other specific gene alterations, thus preventing malignant transformation [115–118]. BRAF mutations in benign nevi are supposed to be associated with induction of senescence [119]. Thus, melanoma is a good indication for the development of drugs targeting the MAPK. Several MAPK kinase inhibitors have recently been developed and these are classified as either BRAF inhibitors or MEK inhibitors as described below.

RAF Inhibitors

The multikinase inhibitor sorafenib (Nexavar®, BAY 43-9006; Bayer AG, Leverkusen, Germany) targets RAF, VEGF receptors (VEGFR) 1, 2, and 3, as well as platelet-derived growth factor (PDGF)-α and PDGF-β, resulting in inhibition of tumor cell proliferation and angiogenesis. In mice xenografts, tumor growth is inhibited [114]; however, complete regression has not been achieved [113, 114, 120].

Unfortunately, in patients, a recent, randomized, placebo-controlled, second-line phase III trial of sorafenib in combination with carboplatin and paclitaxel failed to prove beneficial in terms of overall or progression-free survival in patients with metastatic melanoma. However, the BRAF mutation status of the patient population in the trial was not assessed [121].

More Specific BRAF Inhibitors

More specific inhibitors of mutated BRAF are currently being investigated in clinical trials and have shown more promising results:

- SB590885 (GlaxoSmithKline, Collegeville, Pa., USA) is a selective RAF inhibitor that preferentially targets mutated BRAF rather than wild-type BRAF and CRAF, thus leading to a decreased proliferation of tumor cells. BRAF V600 E mutated cells are inhibited 100-fold more potently by SB590885 than by sorafenib [122].
- RAF-265 (Chir 265; Novartis, Basel, Switzerland) selectively inhibits BRAF, CRAF, and VEGFR 2 and leads to tumor regression in melanoma xenografts. Notably, downregulation of the MAPK pathway has only been obtained in RAS and BRAF mutated melanoma xenograft models [123]. This agent is currently under clinical investigation (http://clinicaltrials.gov/ct2/show/NCT00304525). On the other hand, the selective V600E BRAF inhibitor PLX-4032 and its counterpart PLX-4720 (Plexxikon, Berkeley, Calif., USA) have already shown inhibition of tumor growth preclinically in BRAF V600E mutated cells but not in wild-type cells [124, 125]. In a clinical phase I trial, a reduction of phosphorylated ERK was observed in 3 of 6 patients with V600E BRAF-mutated metastatic melanoma upon treatment with PLX4032 [126]. Furthermore, tumor regression was observed in 5 of 7 patients harboring the V600E BRAF mutation [127].

MEK *Inhibitors*

- PD0325901 (Pfizer, USA) showed responses in 3 out of 22 melanoma patients in a clinical phase I trial; notably, 2 of them had BRAF mutations and 1 had an NRAS mutation. However, phase II trials were suspended due to cases of retinal vein thrombosis [110, 128].
- AZD6244 (ARRY-142886; AstraZeneca, Wilmington, Del., USA), an inhibitor of MEK, induced G1-phase cell cycle arrest, decreased phosphorylated ERK, and showed high cytostatic activity in melanoma xenografts [124, 129, 130]. Despite the xenograft tumor growth was suppressed, the cells remained viable indicating that MEK inhibition alone is not sufficient to induce apoptosis. Tumor regression was achieved, however, using a combination of AZD6244 and docetaxel in melanoma xenografts [129]. A randomized phase II trial of AZD6244 compared to temozolomide reported partial responses in 6 out of 104 patients, 5 of which carried the BRAF mutation (12%) [131]. Clinical trials restricted to patients carrying the BRAF mutation are ongoing.
- NRAS inhibitors, specifically farnesyl transferase inhibitors, i.e. tipifarnib (Zarnestra®, R115777; Johnson & Johnson Pharmaceutical Research and Development; Raritan, N.J., USA) and lonafarnib (Sarasar®, SCH 66336; Schering-Plough Corp., Kenliworth, N.J., USA), showed disappointing results in clinical phase III trials when applied as a monotherapy. Consequently, clinical trials using these inhibitors in combination with chemotherapy are ongoing [110, 132].

At present, it is recommended that targeted therapy be combined with other therapeutic agents in the treatment of metastatic melanoma. In vitro and in vivo studies have shown that monotherapy with one targeted therapeutic agent may lead to tumor stabilization but not to regression [129]. It is assumed that this is because of resistance attained through a switch from BRAF to CRAF signaling [110]. This was demonstrated by one of the first studies of sorafenib, in which monotherapy showed disappointing

results: response in 1 out of 61 patients [133]. However, patients benefitted from sorafenib therapy in combination with dacarbazine when compared to dacarbazine therapy alone [134].

Targeted therapy seems to be a promising therapy option for patients with metastatic melanoma but further investigation is still required.

c-KIT-Targeted Therapy

cKIT is located on chromosome 4q12 and codes for KIT, a tyrosine protein kinase receptor. It belongs to the class III tyrosine kinase receptor family [135–138]. It spans a distance of about 80 kb, including 21 exons [139]. In KIT-expressing melanocytes, KIT controls permanent survival, proliferation, differentiation, and migration functions [140]. Interestingly, the KIT gene originates from a gene duplication of an ancestral PDGFR (PDGF)/KIT locus, followed by a chromosomal duplication during the evolutionary mechanisms involved in the establishment of new cell populations such as neural crest-derived pigmented cells [140].

Why KIT?

cKIT mutations are well known in gastrointestinal stroma tumors (GIST), chronic myeloid leukemia, germ line tumors such as testicular cancer, small cell lung cancer, and mastocytosis [141]. Several reports of positive treatment results in GIST, stable phases of chronic myeloid leukemia, and acute myeloid leukemia [142] prompted the idea to study other cancers' KIT status [143]. In GIST a high frequency of mutations in KIT was detected, leading to constitutive activation of the receptor [144]. Went et al. [145] were the first to detect activating cKIT mutations in melanoma.

As it was found that acrolentiginous and mucosal melanoma showed distinct chromosomal aberrations, several studies focused on and identified cKIT mutations in these melanoma types in particular [102, 103, 105, 106, 146–150]. Targeting the KIT receptor using small-molecule inhibitors such as imatinib showed successful results in certain GIST and was thus evaluated in vitro [147–149, 151] as well as in vivo in clinical phase II studies for acrolentiginous and mucosal melanoma [105, 150, 152–156].

Just recently, NRAS mutations were reported to occur more often in mucosal melanoma than KIT, which is a striking new finding [151].

Imatinib in Melanoma – Successes and Problems

One of the first small molecules targeting KIT was the 2-phenyl-aminopyrimidine, imatinib [157–160]. It shows activity against several receptors such as bcr-abl, KIT, PDGF receptor (PDGFR)-α and PDGFR-β, ABL1, and ABL2 (ARG) [157, 161]. Imatinib functions as a multikinase competitive inhibitor, competing with ATP for the binding site of the tyrosine kinase domain of the KIT receptor. Imatinib can only bind to the nucleotide binding site within the juxtamembrane domain when the DFG (Asp^{810}, Phe^{811}, Gly^{812}) motif is present [162, 163].

Imatinib has been tried in melanoma patients in several clinical studies. The first clinical trial was performed by Ugurel et al. [152] in 2005. Unfortunately, no subgrouping of melanoma took place, nor was the mutation status of KIT considered in a large group. In the 4 cell cultures studied, no mutations were found. Toxicity was outstandingly high in that study. These problems were also present in the study performed by Wyman et al. [154] in 2006; no distinction between melanoma subtypes and neither immunohistochemical nor mutation analysis was performed before running the study. Coincidentally, 1 patient suffered from mucosal melanoma, but no activating mutations were detected. Imatinib treatment was thus insufficient.

Of the 31 patients studied by Kim et al. [153], 1 patient suffered from acrolentiginous melanoma, showing high KIT expression. Imatinib treatment led to a complete response lasting up to 1 year. RNA sequencing revealed a deletion at codon 715 (kinase domain), a splice variant, which was not specific though. Biopsies were taken twice, i.e. before and during treatment. The 1 responding patient showed no alterations in KIT expression during treatment, while the nonresponding patients showed either a reduction, an increase, or no change in KIT expression. Staining was not predictive of therapeutic response [153].

In a clinical phase II study, Heinrich et al. [164] explored the efficacy of imatinib in the treatment of several life-threatening malignancies. Seven melanoma patients were treated, but no effect of imatinib was observed. In 2009, sunitinib showed promising effects in acral and mucosal melanoma in vitro [150].

PDGFR-Targeted Therapy in DFSP

Dermatofibrosarcoma Protuberans

Dermatofibrosarcoma protuberans (DFSP) is the most common sarcoma evolving on the skin; however, it is quite a rare disease with an incidence of less than 1/100,000 per year [165]. DFSPs in most cases are localized on the body trunk (in ~40–70%) in particular at the

presternal region and less frequently on the extremities and head and neck area [166]. Clinically DFSP presents in different forms, often as a plaque on which nodules can evolve. Besides the classical dermatofibroblastic tumor, the fibrosarcomatous variant, FS-DFSP, is seen as its progressive form with increased metastatic potential. The pigmented DFSP, or so called Bednar-tumor, is another subtype which is histologically defined by the occurrence of melanin-containing dendritic cells. Pigmented DFSP, as well as DFSP in general, was reported to be more frequent in the Afro-American population [166, 167]. Specific juvenile form of DFSP is called giant cell fibroblastoma (GCF) where chromosomal alterations are often found [168]. All variants show staining-positivity for CD34 [169] and histological evaluation is always required to make a definitive diagnosis of DFSP [170]. Magnetic resonance imaging (MRI) is helpful for estimation of the tumor's extent [171, 172]. So far, the only staging system that exists was introduced by Ugurel et al. [173], according to the Short German Guidelines for DFSP, suggesting the following: stage I, primary tumor; stage II, including lymph node metastases, and stage III, with distant metastases.

Clinical Development and Presentation

Accretion and development of DFSP takes place over a long time, occasionally years, and diagnosis is often made when the tumor has already progressed and spread. Local infiltration is characterized by asymmetric and horizontal branches, often a few centimeters long, sometimes infiltrating subcutaneous structures such as fascia, muscles, or even bones. Clinically, DFSP can present as skin-colored, brown-to-yellowish, red-tinged, sclerodermiform, or telangiectatic skin [171]. Misdiagnosis as benign skin lesions, such as dermatofibroma and nevi, among others, is another reason for the often late diagnosis. A typical character trait includes the induration and compact consistency of lesions.

Most patients are under 40 years of age. Special risk factors are not known; however, an increased age (>50 years) is associated with a higher propensity for recurrence [174].

Current Therapy Options

Surgery in the early stage is currently the therapy of choice. A challenge is the safety margin (according to the literature it is between 1 and 5 cm), especially in regions such as the presternal or head-neck area where healing with widespread scarring can often be observed. Immunohistochemical staining with CD34 is recommended in order to evaluate the tumor borders in the excised portion. Mohs micrographic surgery allows optimal tumor excision while minimizing the excision of healthy tissue, thus resulting in less complicated wound healing [170, 175, 176].

DFSP is regarded as radiosensitive; thus, postoperative adjuvant radiotherapy is suggested to reduce the risk of local recurrences, especially in patients with narrow and/or positive surgical margins [177–180]. No remarkable benefits are known for conventional chemotherapy [165].

New Molecular Background and Treatment Options

Once the tumor has spread, systemic therapy is required. It has been shown that PDGFR plays a crucial role in the pathogenesis of DFSP. PDGFR is of importance for biological effects such as angiogenesis, cell proliferation, and apoptosis [181].

A translocation of t(17;22) takes place in over 90% of all DSFP patients [182]. The collagen type 1 alpha 1 gene (COLIA1) encoding type I collagen (chromosome 17) and PDGFR (chromosome 22) are located within chromosomal regions affected by this translocation. Fusion of these chromosomes in DFSP leads to constitutively overexpressed PDGFR, causing a continuous autocrine stimulation of tumor cell proliferation. Simultaneously, natural apoptosis is inhibited due to the permanent stimulation of the signal transduction pathway. Already in 1999 Shimizu et al. [183] found a relevant correlation between COLIA1-PDGF-b gene fusion and the inhibition of tumor growth by applying CGP57148B, a PDGF-b inhibitor, in vitro. Interestingly, Shimizu et al. [183] suggested that the COLIA1 part did not contribute to the cellular phenotype. However, the suggested fibroblastic origin of the tumor is attributed to the COLIA1 part [184]. Sjöblom et al. [185] showed STI571-induced (imanitib/Glivec®) apoptosis of DFSP tumor cells but no antiangiogenic effects in in vitro experiments. However, the vascular morphology was reported to be altered upon STI571 treatment.

Who Benefits from Targeting PDGFR?

DFSP is the first dermatological tumor for which signal transduction therapy was approved based on a study of 25 cases of locally spread, recurrent or metastatic DFSP that responded successfully to imatinib [152, 186]. Imatinib is a small multikinase inhibitor targeting PDGFR among other vital kinase receptors such as KIT. The first patient treated with imatinib showed impressive remission of the tumor, which had already metastasized [187].

McArthur and et al. [186] showed that out of 10 patients with locally advanced or metastatic disease the only one without positivity for the t(17;22) translocation did not respond to imatinib; it was thus concluded that the fusion protein is crucial for therapeutic benefit [164]. Interestingly, in contrast to imatinib treatment in GIST, neither high levels of receptor tyrosine kinase activation nor overexpression of the protein was required for successful small-molecule inhibition [186].

In 2005, Price et al. [188] administered imatinib to a child with DFSP, achieving successful tumor treatment. Labropoulos et al. [189] treated a patient with locally advanced recurrent fibrosarcomatous DFSP who had already developed metastases. Imatinib treatment (400 mg daily) led to a complete response [189].

Effects of Imatinib

If imatinib serves as a preoperative treatment option to reduce tumors to a reasonable size, 4–8 weeks after the start of treatment a relevant tumor growth reduction should be seen on MRI. Tumor cells are transformed into less viable hyaline fibers and thus lose vitality [186, 190]. The reduction of vital cells allows a smaller safety margin for the surgical treatment thereof. If no response can be detected at that time, a therapeutic failure is to be considered. Clinical studies for adjuvant imatinib treatment, especially after repeated local recurrences, are still to be performed.

The development of imatinib resistance is challenging; so far, no methods for predicting or preventing this are known [186]. To avoid resistance, other inhibitors such as sunitinib, dasatinib, and nilotinib should be evaluated in clinical trials on DFSP patients.

Conclusion

Imatinib has been shown to target PDGFR in particular, blocking tumor growth and proliferation in DFSP. The striking success of imatinib in DFSP and in cKIT-mutated mucosal or acrolentiginous melanoma is an example for the potential of targeted therapy in skin cancers on one side. On the other side, melanoma perfectly reflects the complexity of pathways resulting in a highly aggressive malignancy. Targeted therapy has recently also yielded antitumor effects in melanoma patients with mutations in the BRAF. Despite several new promising small molecules there are still no therapeutic strategies that reliably increase the survival of patients with advanced disease. Large therapeutic trials including genomic and transcriptional analysis shall identify the subpopulations of skin cancer patients that may profit from the molecules available today. With regard to BCC and SCC the conclusions are similar. Hopefully, intensive basic research together with translational research in the context of well-designed clinical trials will pave the way to a successful and well-tolerated personalized medical management.

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